Group I: Claims 1-4, drawn to a method of detecting molecules expressing a selected epitope in a sample, classified in class 435, subclass 7.1

Group II: Claims 5-11, drawn to a system and kit of detecting molecules expressing a selected epitope in a sample, classified in class 422/435, subclass 61/287.2

The Examiner suggests that the inventions of Groups I and II are distinct because although related as process and apparatus for its practice, the apparatus as claimed can be used to practice another and materially different process, *i.e.*, to detect nucleic acid. Applicants respectfully traverse this rejection.

In order for a restriction requirement to be proper, two criteria must be met (MPEP 803). First, the inventions must be independent and distinct as claimed. Second, there must be a serious burden on the Examiner if the invention is not restricted. Review of the claims as grouped by the Examiner indicates that a search of the published literature that was directed to the process of the present invention, including each of its limitations, would by its design identify literature that would describe any apparatus for using the method of the present invention as described in claims 1-4. Therefore, there would not be a serious burden on the

Examiner if the invention were not restricted. Accordingly, the restriction of the invention is improper and withdrawal of the restriction requirement is respectfully requested.

In an earnest effort to be completely responsive, Applicants elect Group II, claims 5-11 with traverse.

II. Specification

The Examiner suggests that the title of the invention is not descriptive because it does not describe what is claimed based on current claim language. Applicants have amended the title as requested.

III. Information Disclosure Statement

With regard to references AB and AH in the Information Disclosure Statement (IDS), as indicated in the Transmittal Letter sent with the IDS, copies of these books were not provided due to their voluminous nature and the likelihood that the Examiner would have access to these standard reference text books. With respect to reference AH, the publication year which was inadvertently left off from the IDS, is 1998. Please advise if submission of a replacement IDS is required.

IV. Rejection of Claims Under 35 U.S.C. 112, Second Paragraph

Claims 5-11 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Examiner suggests that the claims are vague and indefinite because it is unclear whether or not the molecules expressing a selected epitope are cells as shown in example 1 of the specification. Further, the Examiner suggests that it is unclear what is meant by "a single chain Fv" as it appears that the variable domain of an antibody has light and heavy chains.

Applicants respectfully disagree with the Examiner's suggestion that the claims are vague in terms of defining "molecules" that express a selected epitope. As taught throughout the specification as filed, and in particular at page 14, lines 16-24, and page 14, line 32 through page 15, line 12, by "molecules" it is meant not only to include cells, but also cell extracts and molecules contained within different compartments of a cell. MPEP 2173 clearly states that "Definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of the prior art, and (3) claim interpretation that would be given by one

possessing the ordinary level of skill in the pertinent art at the time the invention was made." The specification as filed clearly teaches the breadth of what is considered to be a "molecule" in the context of the claims. Therefore claims 5-11 are not indefinite as written and filed, and withdrawal of this rejection under 35 U.S.C. 112, second paragraph is respectfully requested.

With regard to the phrase "a single chain Fv", it is respectfully pointed out that throughout the specification it is made clear that by single chain Fv it is meant to be inclusive of the heavy or light chain. See in particular page 6, lines 9-30, and at page 10, line 26 through page 11, line 2 of the specification. As discussed above, the claim language must be read not in a vacuum but in light of the teachings of the specification where it is clearly taught that the single chain Fv can be a light or heavy chain. However, in an earnest effort to advance the prosecution, Applicants have amended the claims to specify that the single chain Fv can be a heavy or light chain. Thus, withdrawal of this rejection under 35 U.S.C. 112, second paragraph, is respectfully requested.

V. Rejection of Claims Under 35 U.S.C. § 103(a)

Claims 5, 6, and 8-11 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Suzuki et al. (1995), in view of

Skerra et al. (1988). The Examiner suggests it would have been *prima facie* obvious to one of ordinary skill in the art to develop a system or kit for detecting a molecule expressing a selected epitope comprising a selected surface to which the epitope binds and an epitope detector comprising a single chain Fv or CDR since the claim language is unclear and Suzuki teaches an antibody that acts to bind an antigen, *i.e.*, the epitope, and an antibody that can have a Fv fragment and thus can bind an antigen, while Skerra et al. teach use of CDR for antigen recognition. The Examiner suggests one would have been motivated to combine these references because the system of Suzuki et al. has a high sensitivity, higher than ELISA. The Examiner further suggests that making a kit or system based on these references would be routine practice to one of skill. Applicants respectfully traverse this rejection.

At the outset, claim 8, and by dependency claims 9-11, have been amended to specify that the kit is used for quantifying molecules expressing a selected epitope. Support for this amendment can be found throughout the specification as filed but in particular at page 5, lines 8-27. Further, the claims have been amended to clarify that by Fv fragment it is meant a single heavy or a light chain Fv attached to an oligonucleotide or a constrained epitope specific CDR attached to an oligonucleotide. Support for

this amendment can be found at page 4, line 33, through page 5, line 6, page 5, line 31, through page 6, line 3, and page 6 wherein Fv fragments are defined.

Suzuki et al. (1995) disclose a method called double determinant immuno-polymerase chain reaction which utilizes two monoclonal antibodies, in which the antigens are sandwiched, and a specific DNA molecule is used as marker. In this method, the first monoclonal antibody to bind the circulating antigen is immobilized instead of the antigen itself. A biotinylated second monoclonal antibody is bound to the antigen and free streptavidin is used to attach a biotinylated DNA to the second monoclonal antibody. The biotinylated DNA complexed with antigen-antibody-streptavidin is amplified by PCR and the products then analyzed by Southern blot analysis.

As pointed out in the specification as filed, the method of Suzuki et al. is limited in that there is no direct correlation between the amount of signal and the amount of protein present, indicating that the method has limited value as a **quantitative** detection method. Although able to detect antigen with high sensitivity, the method of Suzuki et al. is not a quantitative tool.

In contrast, as discussed above, the kits of the present invention are useful in quantifying molecules.

Further, there is no teaching or suggestion by Suzuki et al. with respect to use of a detection means other than antibodies. In contrast, in the kits of the present invention, the epitope detector consists of a single heavy chain or a light chain Fv for a selected epitope attached to an oligonucleotide or a constrained epitope specific CDR attached to an oligonucleotide.

Therefore, the primary reference fails to teach or suggest the limitations of the claims as amended.

The secondary reference cited fails to overcome the deficiencies in teaching of the primary reference. Skerra et al. (1998) disclose an expression system for production of functional Fv fragments in *E. coli*. Nowhere does this paper teach or suggest quantification of molecules expressing an epitope via an epitope detector consisting of a single heavy chain or a light chain Fv for a selected epitope attached to an oligonucleotide or a constrained epitope specific CDR attached to an oligonucleotide.

In order to establish a *prima facie* case of obviousness under 35 U.S.C. 103(a) three basic criteria must be met. MPEP 2143. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to

one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all claim limitations. Clearly, this combination of references fails to teach the limitations of the claims as amended. Therefore, these references cannot establish a case of *prima facie* obviousness under 35 U.S.C. 103(a). Withdrawal of this rejection is respectfully requested.

Claim 7 has also been rejected under 35 U.S.C. 103(a) as being unpatentable over Suzuki et al. (1995), in view of Skerra et al. (1988), as applied to claims 5, 6 and 8-11 above, and further in view of Quentin-Millet et al. (US Patent 4,965,205). The Examiner suggests that it would have been *prima facie* obvious for one of ordinary skill in the art to combine the teachings of Suzuki et al. (1995) and Skerra et al. (1988) with those of Quentin-Millet et al. which teaches detection of an antigen by ELISA using anti-filamentous hemagglutinin antibody, which would be seen by one of skill as the universal epitope detector specified in claim 7.

Applicants respectfully traverse this rejection.

As discussed *supra*, the combined teachings of Suzuki et al. and Skerra et al. fail to teach the limitations of the claims as amended. Thus, while Quentin-Millet et al. may teach detection of

an antigen by ELISA using anti-filamentous hemagglutinin antibody, this secondary reference still fails to overcome the deficiencies in teaching of the primary references towards the independent claims, from which claim 7 depends. Accordingly, this combination of prior art also fails to teach the limitations of the claims, including claim 7, as established under MPEP 2143. Withdrawal of this rejection is therefore respectfully requested.

V. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited. A page has been attached hereto which shows the changes made to the claims and specification and is labeled "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

Respectfully submitted,

Jane Massey Licata
Registration No. 32,257

Date: November 5, 2001

Licata & Tyrrell P.C.
66 East Main Street
Marlton, New Jersey 08053

(856) 810-1515

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The specification has been amended as follows:

Title beginning at line 1 of page 1 has been amended as follows:

~~**A RAPID, SENSITIVE AND QUANTITATIVE METHOD FOR IMMUNO-DETECTION OF EPITOPES ON MOLECULES USING A SINGLE CHAIN FV FOR THE EPITOPE OR A CONSTRAINED EPITOPE SPECIFIC CDR**~~ **METHODS, SYSTEMS AND KITS FOR IMMUNO-DETECTION OF EPITOPES EXPRESSED ON MOLECULES**

Paragraph beginning at line 17 of page 2 has been amended as follows:

Suzuki et al. (Jpn. J. Cancer Res. 1995 86:885-~~88~~ 89) describe a method called double determinant immuno-polymerase chain reaction (double-determinant immuno-PCR) which utilizes two monoclonal antibodies, in which the antigens are sandwiched, and a specific DNA molecule is used as a marker. In this method, the first monoclonal antibody to bind the circulating antigen is immobilized instead of the antigen itself. A biotinylated second monoclonal antibody is bound to the antigen and free streptavidin is used to attach a biotinylated DNA to the second monoclonal antibody. The

biotinylated DNA complexed with antigen-antibody-streptavidin is amplified by PCR. The products are then analyzed by Southern blot analysis.

Paragraph beginning at line 4 of page 6 has been amended as follows:

Fv fragments for selected epitopes can be produced in cells or on microorganisms by use of recombinant DNA technology. For example, Skerra and Pluckthun (Science 1988 240:1038-~~1044~~ 1041) describe an expression system for production of functional Fv fragments in *E. coli*.

Paragraph beginning at line 19 of page 9 has been amended as follows:

In one embodiment, the site on the Fv or CDR to which the oligonucleotides are attached comprises a series of residues which allow the attachment of linkers consisting of chemicals such as heterodimeric coupling reagents or other linkers. These residues provide a uniform binding site for the linker attachment. The linkers attach to this site and also links oligonucleotides to the Fv or CDR. Oligonucleotides may be unmodified or modified. For example, the presence of the amplified oligonucleotide can be enhanced by incorporating a beacon or fluorescent labeled oligonucleotide into the mixture allowing for rapid semi

quantitative assessment of the epitope expressing molecules (~~Ton~~ Tan et al. Chemistry 2000 6:1107-1111; Leone et al. Nucleic Acids Res. 1998 26(9):2150-2155).

Paragraph beginning at line 17 of page 12 has been amended as follows:

The method of the present invention is also useful in the detection of post-translation modifications. PCR and aRNA techniques were originally developed to detect the activity of target genes at the DNA level. These methods have been adopted exclusively in the application of genomics research, sometimes combined with hybridization. Regardless of sensitivity, these methods are not able to detect the post-translation modification at the protein level. Monitoring of such events, however, is very critical since many modifications including, but not limited to, phosphorylation and glycosylation are related to the functional status of the protein. Thus, experiments were performed to demonstrate the ability of the method of the present invention to detect the phosphorylation of the p185 receptor induced by EGF treatment. A signaling model was established in which, upon EGF stimulation, EGFR heterodimerizes with and trans-activates p185, resulting in the phosphorylation of tyrosine residues on the p185 receptor (Qian et al. Proc. Natl Acad. Sci. 1994 ~~15~~ 91:1500-1504).

The A431 cell line, which over expresses EGFR as well as p185 erbB2, was used in these experiments. After EGF stimulation, the p185 receptor in the cell lysate was captured by 1E1, a monoclonal antibody developed against p185erB2/neu. PY99, an IgG2b type of anti-phosphorylated Tyr antibody, was used to detect phosphorylated receptors. A second antibody, anti-IgG2b, coupled with ds-oligo, was used to probe the antigen-antibody sandwich complex. A431 cells stimulated with EGF produced a positive band, which was not observed in cells without EGF treatment. T6-17 cells, however, also showed a positive band, indicating constitutive phospho-tyrosine on the p185 receptor. These data indicate that this method is capable of detecting the functional status of a protein by analyzing its modification. Epitope detectors comprising an Fv or CDR coupled to the ds-oligo can also be used to detect the functional status of the protein.

Paragraph beginning at line 25 has been amended as follows:

The original immuno-PCR used pure antigens in the assay. Later iterations of Immuno-PCR examined mixed antigens (Hendrickson et al. Nucleic Acids Research ~~1999~~ 1995 23(3):522-529) but only showed sensitivity of two to three orders of magnitude higher than ELISA. In a real-world assay with the background comprising a huge variety of non-specific antigens, sensitivity is always limited by the

specificity of the assay. Epitopes bound by the Fvs or CDR fragments are expected to identify larger polypeptides and can be used to identify motifs in supernatants, fluids, extracts of cells or bacteria or any other eukaryotic organism. Further, actual identity of the polypeptides, organic molecules or sugar structures can be determined by computer aided analysis of data bases using the binding of several epitopes by Fvs as a guide. For example, binding by Fv a, d, e, and f would identify a sugar molecule as having side chains a, d, e, and f, and hence belonging to a family of sugars having these same side chains. In this way the present invention allows definition and identification of many, if not all molecules in a cell at any one particular time. Moreover this approach can be used to identify alternative transcriptional forms translated in an active cell or cellular supernatant. This procedure is easily amenable to 1) use with nonradioactive detection methods, 2) microtized liquid handling procedures, 3) low sample volume detection such as "protein chip" analysis and 4) robotization.